Supplementary Materials and Methods

Whole-mount in situ hybridization

Whole-mount *in situ* hybridization was performed as previously described (Kainz et al., 2011). A digoxigenin (DIG)-labeled antisense RNA probe for *Gb-Blimp-1* was used at 1.0 ng/µL and hybridized at 65℃. Double detections of transcripts and proteins were carried out as previously described (Donoughe et al., 2014).

Immunohistochemistry

Antibody staining was carried out according to standard protocols (Patel, 1994) using the following primary antibodies: rabbit anti-Gb-Piwi (Ewen-Campen et al., 2013) 1:300; rabbit anti-Phospho-Smad1/5/8 1:2000 (gift of Dan Vasiliauskas, Susan Morton, Tom Jessell and Ed Laufer, Columbia University, USA); and rabbit or mouse anti-human-Blimp-1 1:300 (Active Motif # 61054 or # 61168, respectively). Secondary antibodies were goat anti-rabbit and goat anti-mouse coupled to Alexa 488 or Alexa 555 (Life Technologies) at a concentration of 1:1000. 5% normal goat serum was used as a blocking solution. DNA was stained with Hoechst 33342 (Sigma) at 1:5000 of a 10mg/ml stock solution.

BMP pathway activation

BMP activation was achieved by injecting recombinant *Drosophila melanogaster* Dpp protein (R&D Systems #519-DP-020/CF) in 4mM HCl to 100 µg/mL into eggs at 30-36h AEL, with 100µg/mL BSA (New England BioLabs #B9001S) in 4mM HCl as the control, as previously described (Donoughe et al., 2014).

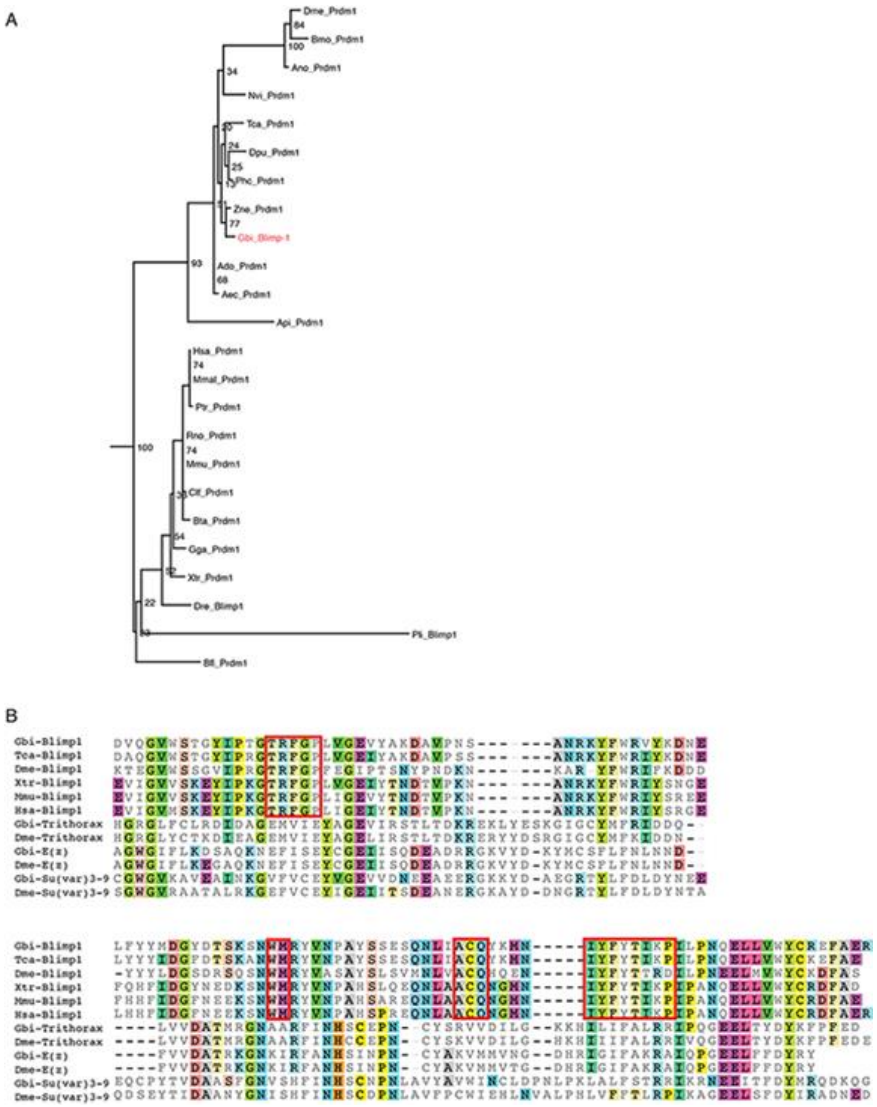

Figure S1. Maximum likelihood tree of Blimp-1/Prdm1 amino acid sequences in selected metazoans and conserved amino acid sequences in the SET domain of Blimp-1/Prdm1 orthologs. (A) Confidence scores labeled on the edges are bootstrap values (1,000 repeats). The distance scale is in raw score values from RaxML. The *G. bimaculatus* sequence reported in this study is indicated in red. Species abbreviations as follows: Gbi, *Gryllus bimaculatus*; Phc, *Pediculus humanus corporis*; Tca, *Tribolium castaneum*; Ame, *Apis mellifera*; Bmo, *Bombyx mori*; Aga, *Anopheles gambiae*; Dme, *Drosophila melanogaster*; Dre, *Danio rerio*; Xtr, *Xenopus tropicalis*; Gga, *Gallus gallus*; Mmu, *Mus musculus*; Hsa, *Homo sapiens*. (B) Sequence alignment of the PR domain of *Blimp-1/Prdm1* in *G. bimaculatus*, *T. castaneum*, *D. melanogaster*, *X. tropicalis*, *M. musculus* and *H. sapiens*, with the SET domain of *Suppressor of variegation 3-9* in *G. bimaculatus* and *D. melanogaster* and *Enhancer of Zeste* in *G. bimaculatus* and *D. melanogaster*, and Trithorax factors in *G. bimaculatus* and *D. melanogaster*. Sequence alignment was made using ClustalW2 in Geneious. Red boxes indicate motifs conserved in most PR domains but absent from most SET domains.

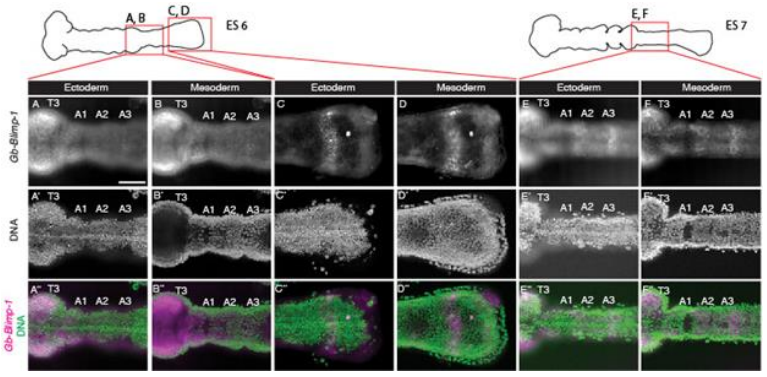

Figure S2. The dynamic expression patterns of *Gb-Blimp-1* during the posterior elongation process. *Gb-Blimp-1* expression in ectoderm and mesoderm is shown in two different focal planes. Colorimetric *Gb-Blimp-1* expression signal photographed under white light was converted to pseudo-fluorescent images using Photoshop (top row) in order to superimpose these data with Hoechst signals (middle row). Merged images (bottom row) show that *Gb-Blimp-1* transcripts are expressed in mesodermal area with signal intensity.

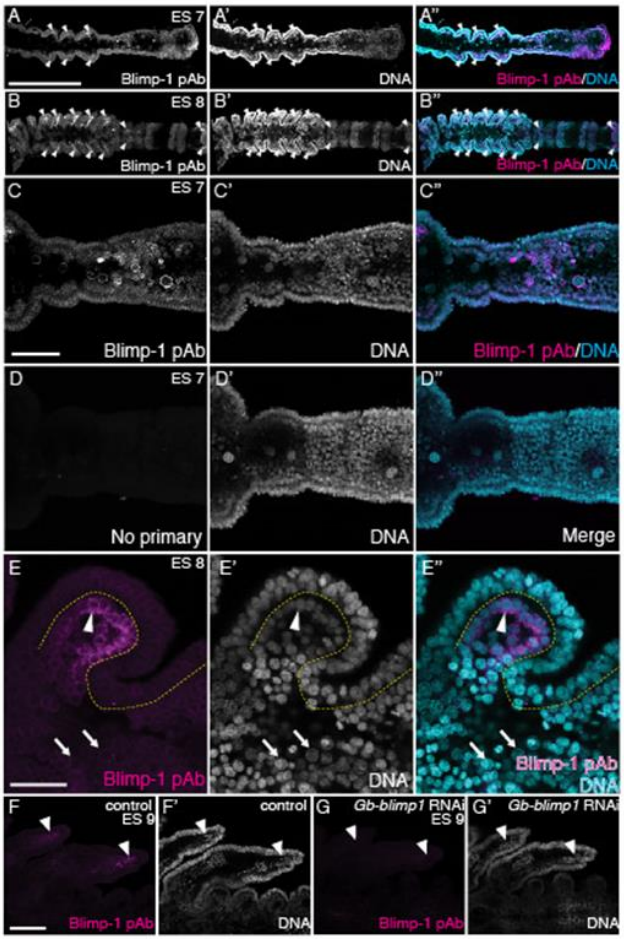

Figure S3. Blimp-1 protein expression in *G. bimaculatus* embryos largely overlaps with the expression pattern of *Gb-Blimp-1* transcripts. Embryos stained with a cross-reactive *H. sapiens* anti-Blimp-1 polyclonal antibody at ES7 and ES8 as PGCs are emerging, show that Blimp-1 protein expression is similar to that of *Gb-Blimp-1* transcripts (Figs. 1, 2) in the mesodermal tissues. (A-A'') Blimp-1 protein is weakly distributed throughout the whole embryo, but higher signal intensity (arrowheads) is found in mesodermal tissues of leg primordia. (C-C'') Higher magnification images of the anterior abdominal regions show Blimp1 positive cells are distributed in the abdominal mesoderm but not in the dorsally located ectoderm region. (D-D'') Secondary-only controls imaged at the same confocal settings as the micrographs shown in (C-C'') show that the Blimp-1 signal is specific to the *H. sapiens* anti-Blimp-1 polyclonal antibody. (E-E'') Anti-Blimp-1 signals are detected in the mesodermal region of T3 appendage primordia (arrowheads) but not in the medial mesoderm of the thoracic segment (arrows). Yellow dotted line indicates the boundary between thoracic mesoderm and ectoderm. (F-G') Anti-Blimp-1 signals are abolished by *Gb-Blimp-1* RNAi (80%, n=5), which reduces *Gb-Blimp-1* transcript levels (Fig. 3A), indicating the specificity of this antibody for *G. bimaculatus* Blimp-1 protein.

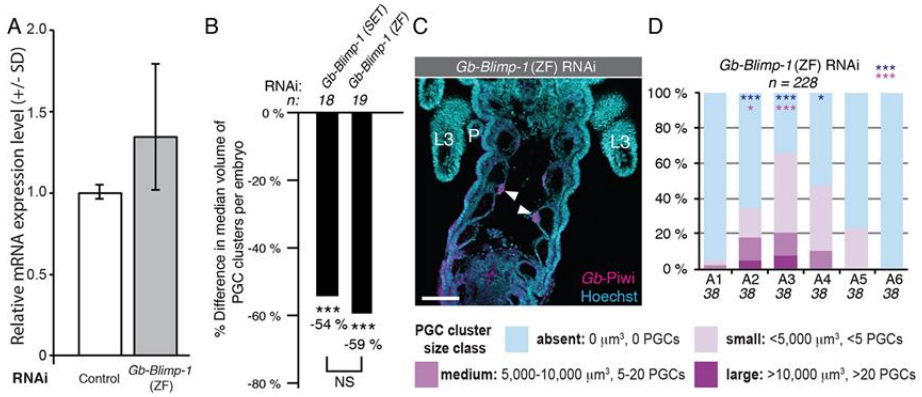

Figure S4. PGC quantification in *Gb-Blimp-1* (ZF) RNAi embryos. (A) qPCR shows that *Gb-Blimp*-1 transcript levels are not detectably reduced by *Gb-Blimp-1* (ZF) RNAi. (B) Percent difference in the median total volume of PGCs clusters per embryo between *Gb-Blimp-1* RNAi embryos using dsRNA against the SET domain or zinc-finger (ZF) domain. Despite the lack of detectable reduction in *Gb-Blimp-1* transcripts under *Gb-Blimp-1* (ZF) RNAi treatment (A), this treatment significantly reduces the median volume of PGC clusters per embryo, to the same extent as the reduction caused by the *Gb-Blimp-1* (SET) RNAi treatment, which does reduce *Gb-Blimp-1* transcript levels (Fig. 3A). No statistical differences in PGC cluster volume were found between *Gb-Blimp-1* (SET) RNAi and *Gb-Blimp-1* (ZF) RNAi embryos. (C) Abdominal segments A1-A6 in representative 4d (AEL) embryos from *Gb-Blimp-1* RNAi (ZF) treatment. PGCs are identified with anti-*Gb*-Piwi antibody (magenta; arrowhead). (D) PGC quantification per segment at 4 dAEL for *Gb-Blimp-1* (ZF) RNAi embryos. Blue asterisks indicate significance of presence/absence of PGC clusters compared with controls; pink asterisks indicate significance of size differences of PGC clusters compared with controls. A Mann-Whitney test was used to calculate significance in B and D: *P < 0.05, **P < 0.01, ***P < 0.001.

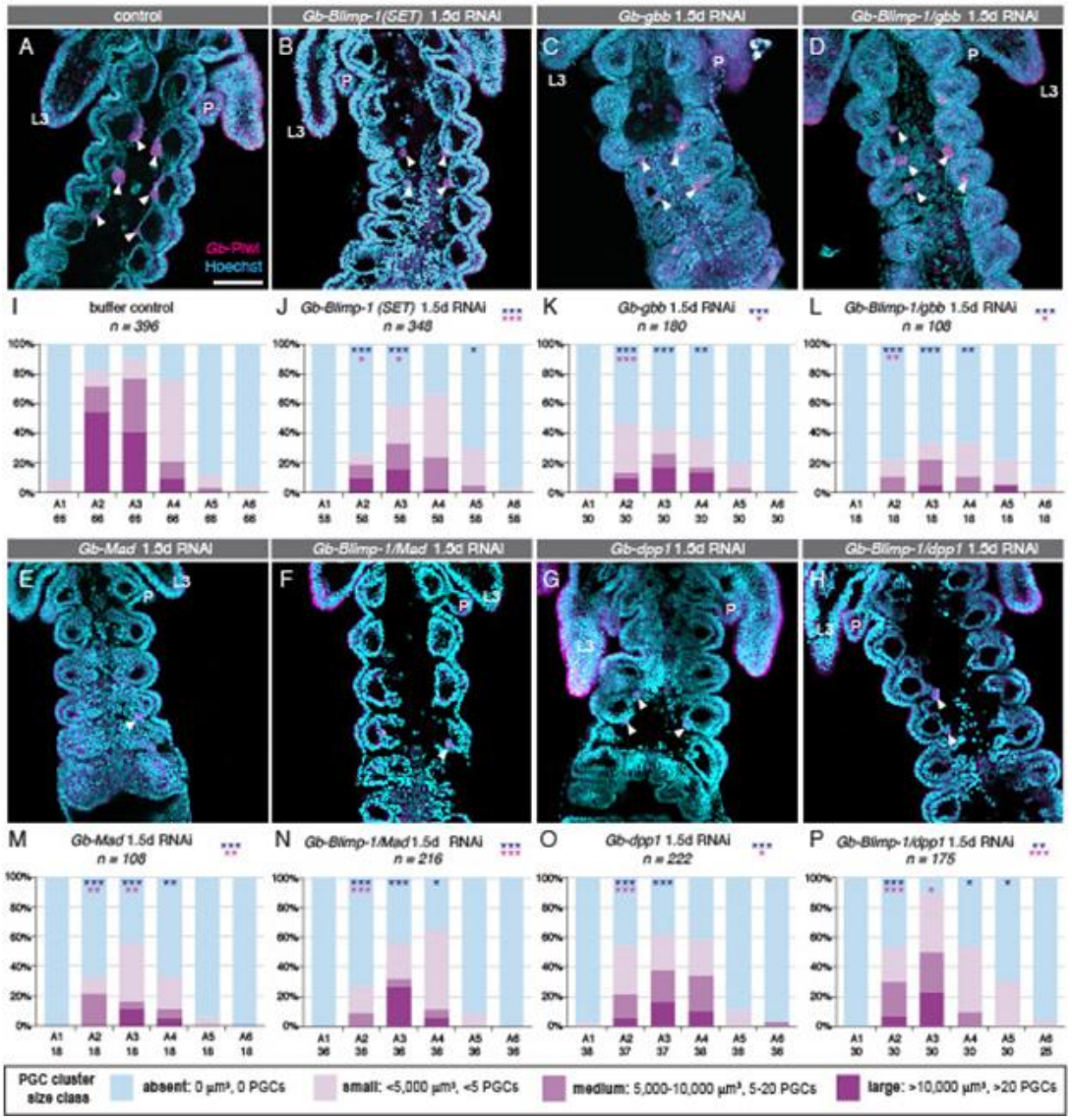

Figure S5. PGC quantification in single and double RNAi embryos for *Gb-Blimp-1* and BMP signaling pathway genes. (A-H) Abdominal segments A1-A6 in representative 4d AEL embryos from the indicated RNAi treatments. PGC clusters (arrowheads) are identified with an anti-*Gb*-Piwi antibody (magenta). (I-P)) PGC quantification per segment at 4 d AEL for embryos from the indicated RNAi treatments. Blue asterisks indicate significance of presence/absence of PGC clusters compared with controls; pink asterisks indicate significance of size differences of PGC clusters compared with controls. A Mann-Whitney test was used to calculate significance: *P < 0.05, **P < 0.01, ***P < 0.001.

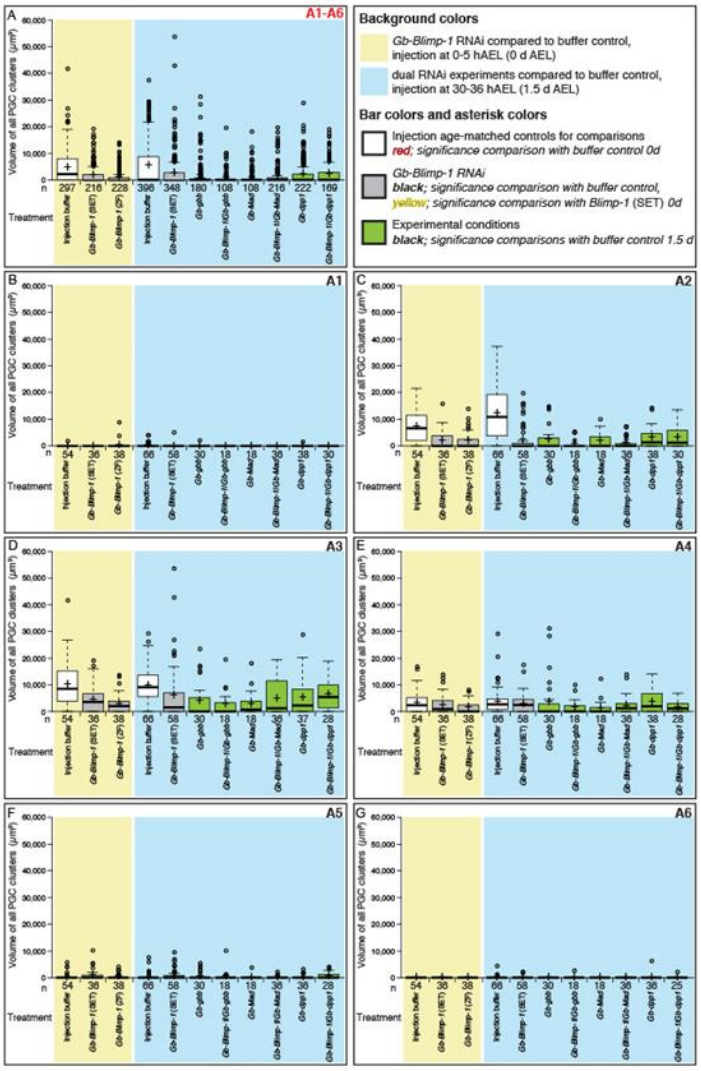

Figure S6. Distributions of all PGC cluster sizes in all RNAi treatments. Box-and-whisker plots showing the distribution of volumes of all individual PGC clusters, including absent clusters (volume = 0 μm^3) from all embryos scored in *Gb-Blimp-1* RNAi experiments (yellow background) and double RNAi experiments, in which embryos were co-injected with dsRNA for both *Gb-Blimp-1* and a BMP signaling pathway member (blue background). Boxes: interquartile range (IQR); thick black line: median; thin black line: whiskers extending to data points that are less than 1.5 x the IQR away from 1st / 3rd quartile. (A) Distributions of volumes of all clusters scored in abdominal segments A1–A6. (B-G) Distributions of volumes of all clusters scored in each abdominal segment, A1 through A6 respectively. A Mann-Whitney test was used to calculate significance in comparison to control, and a Steel-Dwass test, a non-parametric post-hoc test for multiple pairwise comparisons, was used for comparisons among RNAi treated embryos. NS; not significant, *P < 0.05, **P < 0.01, ***P < 0.001. These data are summarized in Fig. 3B (significance indicated by blue asterisks), Fig. 5 and Fig S4D (significance indicated by blue asterisks). Numbers in italics underneath each bar indicate sample size.

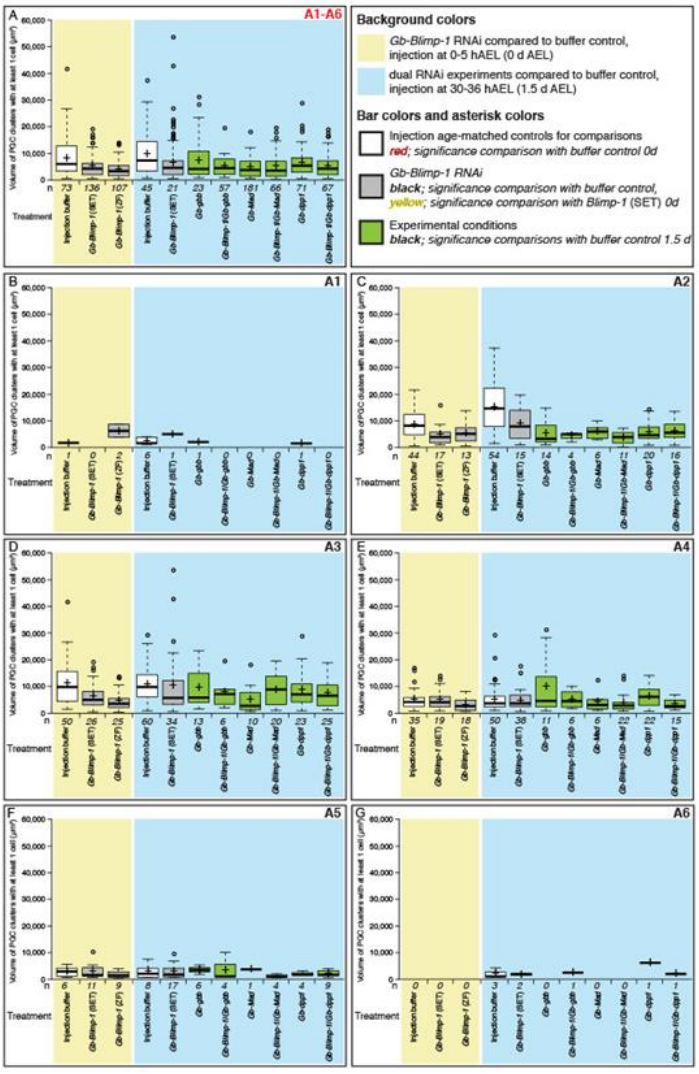

Figure S7. Distributions of non-zero PGC cluster sizes in all RNAi treatments. Box-and-whisker plots showing the distribution of volumes of individual PGC clusters with volume > 0 μm^3 from all embryos scored in *Gb-Blimp-1* RNAi experiments (yellow background) and double RNAi experiments, in which embryos were co-injected with dsRNA for both *Gb-Blimp-1* and a BMP signaling pathway member (blue background). Boxes: interquartile range (IQR); thick black line: median; thin black line: whiskers extending to data points that are less than 1.5 x the IQR away from 1st / 3rd quartile. (A) Distributions of volumes of all clusters scored in abdominal segments A1–A6. (B-G) Distributions of volumes of all clusters scored in each abdominal segment, A1 through A6 respectively. A Mann-Whitney test was used to calculate significance in comparison to control, and a Steel-Dwass test, a non-parametric post-hoc test for multiple pairwise comparisons, was used for comparisons among RNAi treated embryos. NS; not significant, *P < 0.05, **P < 0.01, ***P < 0.001. These data are summarized in Fig. 3B (significance indicated by blue asterisks), Fig. 5 and Fig S4D (significance indicated by blue asterisks). Numbers in italics underneath each bar indicate sample size.

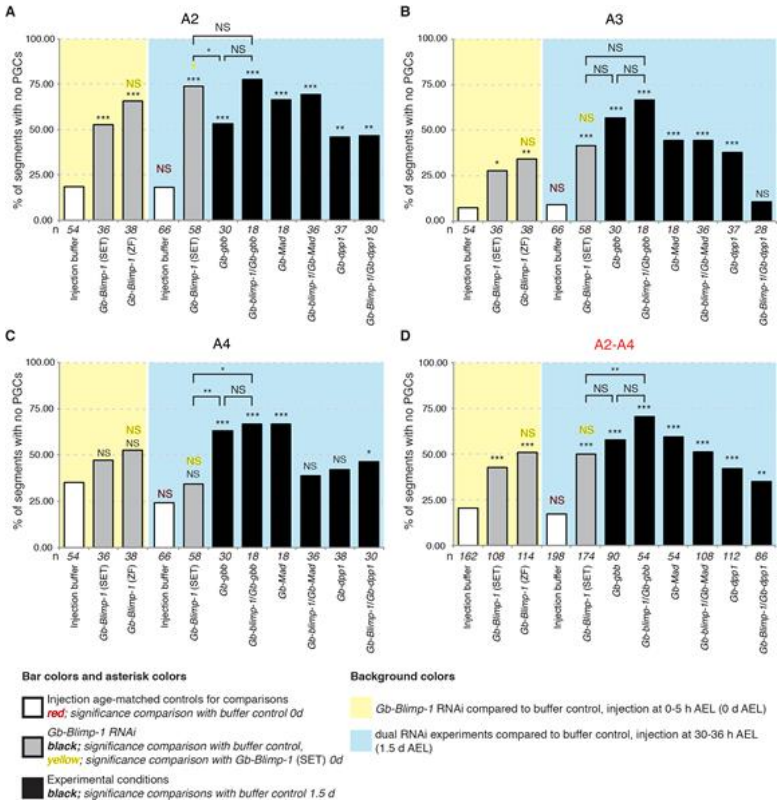

Figure S8. Proportion of abdominal segments A2-A4 with no PGCs at all in *Gb-Blimp-1* RNAi embryos. Proportion of segments A2-A4 with no PGCs at all in *Gb-Blimp-1* RNAi embryos compared with controls (light yellow background) and double RNAi embryos compared with controls (light blue background). Chi-squared test was used for statistical analysis: NS = not significant; *P < 0.05, **P < 0.01, ***P < 0.001. Numbers in italics underneath each bar indicate sample size.

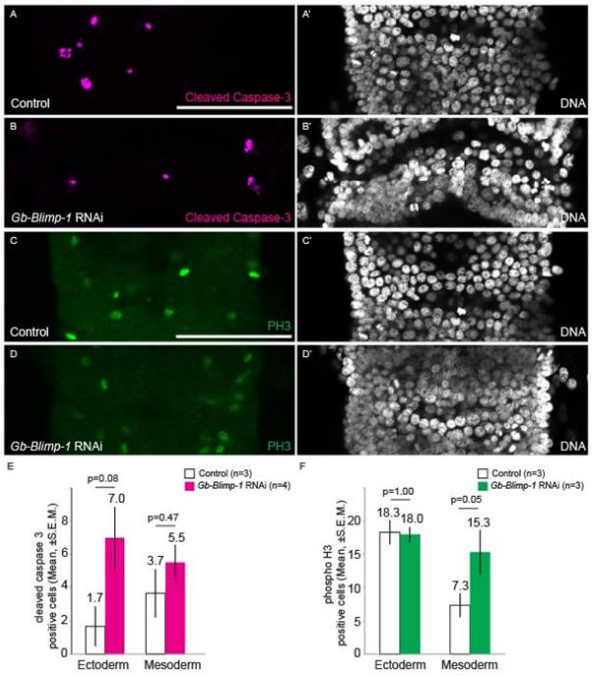

Figure S9. *Gb-Blimp-1* RNAi does not affect cell apoptosis and mitosis in both mesoderm and ectoderm. (A-B') 2.5 hAEL embryos from *Gb-Blimp-1* RNAi and Control with anti-Cleaved Caspase-3 and Hoechst to visualize apoptosis cells. (C-D') 2.5 hAEL embryos from *Gb-Blimp-1* RNAi and Control with anti- Phosphorylated Histone-3 and Hoechst to visualize mitotic cells. (E, F) The mean number of apoptotic cells or mitotic cells per embryo in the ectoderm and mesoderm tissues of abdominal region A2-A4, showing no statistically significant difference. Error bars represent the SEM. The p value is derived from the Mann-Whitney U test (non-parametric test). Scale bars 100 μm in A and C applies to all panels.

Table S1. Primers for used gene cloning and qPCR. Indicates whether dsRNA fragment synthesized was targeting SET domain or ZF domain.

Gene name / primer name	Gene region targeted (1)	length (bp)	Forward primers (5'-3')	Reverse primers (5'-3')
Gb-Blimp-1	ORF, SET domain	327	CAAGGCGTATGGAGCACTGG	CCTTTCGGCAAACTCTCTGC
Gb-Blimp-1	ORF, ZF domain	342	CCAGCTGTCCAACCTCAAG	GCGCTGCTGTACTTCTTGCTG
Gb-Blimp-1, qPCR	ORF (Exons boundary)	118	TCCGAATCCCGATGTGAG	CGTTATGCAGTGTGTTTGTTGTG

Injectant	**Injection age (h AEL)**	**dsRNA Fragment (1)**	**Concentration**	**# embryos injected**	**% (#) embryos survived injection (2)**	**# embryos scored for PGCs in segments A1-A6 (3)**	**% (#) scored embryos with no PGCs in entire embryo (4)**	**# embryos scored for PGCs in segments A2-A4 (5)**	**% (#) scored embryos with PGCs absent in ≥4 of 6 A2-A4 hemisegments (5)**	**% (#) scored embryos with fewer PGCs in A2-A4 than lower quartile of control embryos (5)**
Injection buffer	0-5	–	–	194	84.5 (164)	27	3.7 (1)	27	7.41 (2)	25.9 (7)
Injection buffer	30-36	–	–	94	90.4 (85)	33	6.1 (2)	33	9.1 (3)	24.2 (8)
Gb-Blimp-1 dsRNA	0-5	ORF (SET)	4.0 µg/µL	206	84.5 (174)	18	0.0 (0)	18	38.9 (7)**	61.1 (11)*
Gb-Blimp-1 dsRNA	0-5	ORF (ZF)	4.0 µg/µL	205	70.2 (144)***	19	10.5 (2)	19	36.8 (7)**	78.9 (15)***
Gb-Blimp-1 dsRNA	30-36	ORF (SET)	4.0 µg/µL	194	84.5 (164)	29	20.7 (6)	29	37.9 (11)**	79.3 (23)***
Gb-gbb dsRNA	30-36	ORF	4.0 µg/µL	55	70.9 (39)**	15	40.0 (6)**	15	46.7 (7)**	80.0 (12)***
Gb-gbb/Gb-Blimp-1 dsRNAs	30-36	ORF / ORF (SET)	4.0 µg/µL each	50	68.0 (34)***	9	44.4 (4)**	9	66.7 (6)***	77.8 (7)**
Gb-Mad dsRNA	30-36	ORF	4.0 µg/µL	208	86.1 (179)	9	44.4 (4)**	9	44.4 (4)*	100 (9)***
Gb-Mad/Gb-Blimp-1 dsRNAs	30-36	ORF / ORF (SET)	4.0 µg/µL each	60	78.3 (47)*	18	16.7 (3)	18	38.9 (7)*	83.3 (15)***
Gb-dpp1 dsRNA	30-36	ORF	4.0 µg/µL	49	85.7 (42)	19	21.1 (4)	19	31.6 (6)*	68.4 (13)**
Gb-dpp1/Gb-Blimp-1 dsRNAs	30-36	ORF / ORF (SET)	4.0 µg/µL each	59	81.4 (48)	15	0.0 (0)	14	14.3 (2)	85.7 (12)***
BSA	30-36	–	100 µg/ml	98	93.9 (92)	–	–	–	–	–
Dm-Dpp	30-36	–	100 µg/ml	91	93.4 (85)	–	–	–	–	–

Table S2. RNAi and injected protein used for functional *Gb-Blimp-1* experiments. 1. Region of the injected dsRNA was synthesized part of the open reading frame (ORF). **2.** Embryos were scored as surviving the injection at the time of 48 hours following injection. **3.** Quantitative PGC scoring was performed embryos at 4-4.5 days after egg laying by staining with the germ cell marker anti-*Gb*-Piwi. Remaining embryos of RNAi and injected protein were used for qPCR, pMad, in situ hybridization, apoptosis and proliferation experiments. **4.** Includes only embryos in which all hemisegments of A1-A6 were scored. **5.** Includes only embryos in which all hemisegments of A2-A4 were scored. Asterisks indicate statistical significance relative to controls, calculated using a Pearson Chi-Squared t test. ***P < 0.001, **P < 0.01, and *P < 0.05.

Supplementary References

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